HUNTINGTON MEDICAL RESEARCH INSTITUTES
NEUROLOGICAL RESEARCH LABORATORY
734 Fairmount Avenue
Pasadena, California 91105

Contract No. NO1-NS-8-2388

QUARTERLY PROGRESS REPORT

March 1 - June 30, 1998

Report No. 2

"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

William F. Agnew, Ph.D.
Leo A. Bullara, B.A.
Ted G.H. Yuen, Ph.D.
Douglas B. McCreery, Ph.D.
Albert S. Lossinsky, Ph.D.

ABSTRACT

In this study we continued to address the problem of lymphocyte accumulation near pulsed tips of microelectrodes. While the presence of significant numbers of lymphocytes near the tip sites was strongly correlated with whether or not the electrode was pulsed, the density of the lymphocytes was not strongly correlated with any of the stimulus parameters that we tested. Lymphocytes disappeared when the animals were sacrificed 2 or 4 days after the stimulation or when stimulation was continued for 7 hrs/day for 4 days. Microcavitations associated with electrode tracks were prominent and may be related to procedural changes made since our QPR #10, 1997. Another unexpected observation was frequent gliosis both along the shaft and at the tips of pulsed and unpulsed electrodes. Since uninsulated stabilizing shafts had no associated gliosis, some type of chemical or contaminant emanating from the Epoxylite insulation appears to be implicated.

INTRODUCTION

In the past quarter we have continued our investigation of the limits of safe stimulation of the cat's sensorimotor cortex using microelectrodes. Specifically, we are continuing our efforts to determine the cause(s) and extent of lymphocytic accumulation at the tips of pulsed arrays of microelectrodes.

METHODS

Microelectrodes. The intracortical arrays contained 7 discrete activated iridium microelectrodes and in the last 5 experiments, 3 longer shafts were added as inactive stabilizing pins and for improved orientation at autopsy. The shaft thickness of the electrodes was 32 μm and the geometric area of the exposed tips was 500 or 2000 μm^2. The arrays are integrated with a 16-pair percutaneous connection that also includes a large indifferent platinum electrode, a Ag/AgCl reference electrode and connections for the pyramidal tract recording electrode and reference electrode. The complete assembly is soaked in deionized water for 96 hours, then sterilized with ethylene oxide before implantation.

Surgical Procedure. The arrays were implanted into seven young adult cats of either sex, using general anesthesia and aseptic surgical technique. The animal's head was mounted in a stereotaxic holder, the scalp and muscles were reflected in a midline incision, and the pericruciate gyri (sensorimotor cortex) exposed. The frontal air sinus was partly filled with cranioplasty. The percutaneous connector was mounted to the skull with stainless steel screws and methacrylate bone cement. A macrostimulating electrode was placed on the dura over the postcruciate gyrus, and a recording electrode was implanted into the pyramidal tract through a small burr hold over the cerebellum. The large pyramidal tract potential evoked from the surface of the postcruciate gyrus was used to guide the recording electrode into the tract. The recording electrode was then sealed to the skull using methacrylate bone cement. A small flap, slightly larger than the array's superstructure, was made in the dura over the postcruciate cortex, and the array of microelectrodes was inserted using a vacuum-operated electrode inserted attached to the stereotaxic carrier. In this series of animals, we did not suture the dura over the array, but in recent animals (IC-161 and thereafter, Table 1), we covered the array with a sheet of perforated artificial dura (silastic sheeting). The cortex was then covered with gelfoam and the bone flap replaced and sealed with cranioplasty.

At least 45 days after implantation, the 7-hour test stimulation regimen was conducted, with the animal lightly anesthetized with Propofol. Before and after the test stimulation, the response evoked by the intracortical microelectrodes was recorded from the pyramidal tract. By this method, we have determined that the Propofol anesthesia does not reduce the amount of neural activity evoked by the microelectrodes and that it does not induce an elevation of the electrical threshold of the cortical neurons. Most stimulation regimens were 7 hours in duration, but in two cases (#'s 171 and 172) it was extended to 7 hrs/day for 4 days to assess the effect of chronic stimulation on the lymphocytic accumulation. The electrodes were pulsed continuously with charge-balanced, controlled-current, cathodic-first pulses. The pulse duration was 150 or 400 μsec/ph. In all cases, 5 microelectrodes were pulsed and two were left as unpulsed controls. To assess the role of charge density on neural damage and/or the lymphocytic accumulation, the exposed surface area was either 500 or 2000 μm^2. In most animals, the 5 microelectrodes were pulsed sequentially (interleaved) except for animal #'s 170 and 172, which were pulsed in the simultaneous mode. The stimulus parameters for each animal are listed in Table 1.

TABLE I
INTRACORTICAL MICROSTIMULATION
AND LYMPHOCYTE ACCUMULATION

ANIMAL #	DURATION OF STIMULATION (HRS)	POST-STIM. TIME	STIMULATION PARAMETERS*					LYMPHOCYTES AT TIP**						
			MODE	FREQ. (Hz)	CURRENT CH/PH		CHARGE DENSITY ($\mu C/cm^2$)	UNPULSED		PULSED				
					(μA)	(nC)								
166	7	45 min.	Interlv.	25	16	2.4	480	1 0	2 0	3 ++	4 +++	5 +	6 +	7 +
167	7	2 days	Interlv.	50	26.5	4.0	800	1 0	2 +	3 +	4 +	5 0	6 0	7 0
168	**Implanted; Stimulation Pending**													
169	7	4 days	Interlv.	50	26.5	4.0	800	3 0	4 0	1 0	2 0	5 0	6 0	7 0
170	7 hrs/day 4 days	45 min.	Simult.	50	26.5	4.0	800	2 +	4 0	1 +	3 0	5 +	6 +	7 0
171	7	45 min.	Interlv.	50	26.5	4.0	200	4 0	5 0	1 +++	2 +++	3 +++	6 +++	7 +++
172	7 hrs/day 4 days	45 min.	Simult.	50	26.5	4.0	200	1 0	7 +	2 +	3 +	4 +	5 0	6 0
173	7	45 min.	Interlv.	50	26.5	4.0	200	1 +	4 +	2 ++	3 ++	5 +	6 +	7 +
174	**Implanted; Stimulation Pending**													
175	**Implanted; Stimulation Pending**													

*Electrode tip surface areas were 500 μm^2 in animals 166-170; in 171-13 they were 2000 μm^2

**Lymphocyte code: + = <50; ++ = 50-100; +++ = >100 lymphocytes in any one histologic section.

Histology. Most animals were perfused within 45 minutes following termination of stimulation. However, 2 animals were perfused at 2 days and four days (#'s 167 and 169). The cats were anesthetized with Nembutal and perfused through the ascending aorta with ½ strength Karnovsky's fixative (animal IC-158 was perfused with buffered paraformaldehyde fixative).

Histologic evaluations were carried out on serial 8 μm thick paraffin sections cut in the horizontal plane (perpendicular to the electrode shafts). The sections included both implanted (left) and unimplanted (right) cruciate gyri. While we considered the evaluation of the tissue at the pulsed tips to be most critical, serial sections enabled observation of histologic alterations along the entire shaft. At 100 μm intervals below the pial surface, we recorded the presence of any inflammatory cells, hemorrhage, cavitation, scarring, gliosis, edema, sheath thickness, as well as the condition of the neurons, the appearance of blood vessels in the area at these levels.

RESULTS

Findings. Figure 1 shows the seven electrode tracks of a seven electrode array (without the recently added 3 stabilizing electrodes) at a depth of 320 μm. Figure 2 shows a higher magnification illustrating the thin sheath around the electrode track and normal-appearing neurons in the adjacent neuropil.

Acute inflammation was not present at any electrode site in the series. However, in three instances, neutrophils were present in a vascular "cuffing" phenomenon near some tracks, and, infrequently, occasional neutrophils were present among the lymphocytes and glial cells at electrode tip sites. Hemorrhage accompanied five of the 49 electrode tracks and these were present at various depths along the shafts, above the tip sites. In no case was a hemorrhage greater than 100 μm in length and none showed intact erythrocytes but rather, fragmenting cells still retaining their characteristic pink color.

Cavitations adjoining the tracks were present at 32 of the 49 track sites; however, most of the cavitations were small (60 to 70 μm in greater dimension) but occasionally reached a length of 200 to 220 μm. These appeared as non-filled cavities containing loosely aggregated macrophages. Often, these cells had engulfed erythrocytes which, when observed, were already degraded into hemosiderin. Connective tissue elements had not yet appeared to begin the resolution of these sites into scar tissue (Figure 3).

Scars were present at various depths beside 11 tracks. Rarely, these appeared to be the result of electrode movement but generally they appeared to be the result of an earlier hemorrhage which had resolved into a compact scar accompanied by few blood vessels. Most scars were not at the tip sites but were located randomly along the shafts with some as superficial as 100 and 200 μm below the pia.

Gliosis was discontinuous along the tracks of Epoxylite-insulated microelectrodes. With the exception of 2+ (50 to 99 cells) or 3+ (100 or more) accumulations of glial cells at or near some entry or tip sites, they were virtually always 1+ or absent at various depths and showed no pattern with respect to location. All glial gradings were based on estimates as follows: 1+ = 1 to 49 cells in the histologic section through the track; 2+ = 50 to 99 cells; 3+ = 100 or more cells. Gliosis at the tips usually took the form of globular clusters whether or not lymphocytes were present and irrespective of pulsing (Figure 4). Often gliosis coexisted with globular accumulations of lymphocytes (Figure 5A-D - color micrograph). Note in Figure 5-C, a heavy perivascular lymphocytic cuffing with only occasional penetration of the gliotic mass at the tip of the track. *Gliosis was never observed along the tracks of any of the 9 noninsulated stabilizing shafts.*

Edema was a rare finding and was confined to small areas surrounding two of the electrode tracks of animal #167. These areas consisted of slight pallor in the stained sections. Vascular hyperplasia and hypertrophy were present near 16 tracks from seven animals; four of these were unpulsed and in all instances the phenomenon was present at various depths but never at the level of the tip site. Aside from incomplete fixation in two cortical samples and only occasional flattened neurons at various levels near almost all electrode tracks, the neurons appeared normal, including those near the tips of pulsed and unpulsed electrodes.

Stimulus parameters and accumulation of lymphocytes at electrode tips. Figure 5 shows an electrode tip area with no accumulation, or even the presence of a single lymphocyte near this nor any of the other 6 electrodes in the array (animal #161). In contrast, large globules of lymphocytes are shown in Figure 6A-D. (Table I).

We performed a multivariate statistical analysis of the accumulation of the lymphocytes around the tips of the intracortical microelectrodes implanted in cats IC149, 150, 153, 154, 156, 158, 161, 162, 166, 171,173. This series includes animals for which the histologic results were

presented in Quarterly progress report #10 of our previous contract, as well as those of the current series (Table I). In all of these cats, 5 of the 7 microelectrodes were pulsed for 7 hours and the cat was perfused for histologic evaluation within 45 minutes after the end of the stimulation. The stimulus was a continuous train of charge-balanced, cathodic-first pulse pairs. The charge per phase ranged from 2.4 to 8 nC/phase. The pulsing rate ranged from 25 to 200 Hz. The pulse duration was either 150 or 400 μsec/phase. The charge density ranged from 200 to 1600 $\mu C/cm^2$. Charge density was manipulated independently of charge per phase by varying the active surface areas of the microelectrode tips. In cats 171, 172 and 173, the tip areas were 2,000 μm^2,+/15%. In the other animals, the tips areas were 500 μm^2, +/- 15%.

A number of statistical tests were performed, using the UNIMULT statistical package. The charge per phase and the pulsing rate were treated as ordinal (continuous) independent variables. The question of whether the particular electrode was pulsed (*"pulsed/not pulsed"*) was treated as a nominal independent variable. Lymphocyte accumulation around the electrode tips was treated either as a ranked dependant variable with 4 categories (0,1,2,3 as described above, *"lymphocyte density"*) or as a nominal variable (whether or not there were more than 50 lymphocytes (grade 2 or 3) in the tissue surrounding the electrode's tip site, *"many/few lymphocytes"*).

For each of the tests described below, we ran simulations to verify that the tests could detect any real effect of the test variable on lymphocyte density, after the correlated independent variables had been partialed out by the sequential analysis.

The strongest, and highly significant, correlation was between the independent variable *pulsed/not pulsed*, and the dependent variable *many/few lymphocytes*. (Chi-squared test, e=0.8, p<.0001) . The square of the effect size (e^2 or R^2) can be interpreted as a conditional variance. Thus e^2= 0.64 indicates that 64% of the variance in the variable *many/few lymphocytes* is attributable to the pulsing. This is a large part of the total variance, but it also indicates that some other factor or factors make a significant contribution to the variance.

However, there was little correlation between the *lymphocyte density* and the other independent variables tested. There was a small, non-significant positive correlation between *charge per phase* and *lymphocyte density* , after the variable *pulsed/not pulsed* had been partialed out (Sequential linear model analysis, R=0.17, p=0.2). There was virtually no correlation between *lymphocyte density* and *pulse rate*, after the variables *pulsed/not pulsed* and *charge per phase* had

been partialed out (Sequential linear model analysis, r=-0.05, p>0.5). Similarly, there was very little correlation between *lymphocyte density* and *pulse duration*, after the variables *pulsed not pulsed* and *charge per phase* had been partialed out (Sequential linear model analysis, R=0.12, p>0.5). Finally, there was little effect of *charge density*, after the variables *pulsed/not pulsed* and *charge per phase* had been partialed out. (Sequential linear model analysis, r=0.12, p=0.3). Thus, while the presence of significant number of lymphocytes near the tip sites is strongly correlated with whether or not the electrode was pulsed, the density of the lymphocytes was not strongly correlated with any of the stimulus parameters that we tested.

Note, however, that lymphocytic accumulation dissipates when the post-stimulation time before sacrifice is increased to 2 or 4 days or when the stimulation is continued for 7 hrs/day for 4 days (Table I).

DISCUSSION

This study confirms our previous observation that accumulation of lymphocytes is associated with electrical stimulation but in this study we were not able to ascribe the density of the aggregates to a *specific* electrical parameter. This study did demonstrate that the aggregation of lymphocytes is transitory, i.e., it disappears with cessation of stimulation and that it also disappears with chronic (7 hrs/day for 4 days) stimulation. One explanation for this apparent paradox is that an electrochemical product is produced and is adsorbed onto the electrode surface and is later removed by prolonged pulsing of the electrode. An alternative explanation is that with continuous, intensive stimulation there is a depletion of the source (neurons or glial cells?) of lymphocyte-attracting cytokines.

The increase in the incidence of cavitations resulting from microhemorrhages in this report is in striking contrast to our findings as reported previously (QPR #10, 1997) in which cavitations at microelectrode tips were virtually nonexistent. This difference may be due to a change in our insertion procedure during the past year. In order to obtain better electrode alignment, the electrodes were inserted using a vacuum-operated electrode holder mounted on the stereotaxic frame. The animal was hyperventilated and the respirator turned off during insertion to minimize movement of the brain. In our next series, we will return to manual insertion using padded forceps to grasp the array and cable.

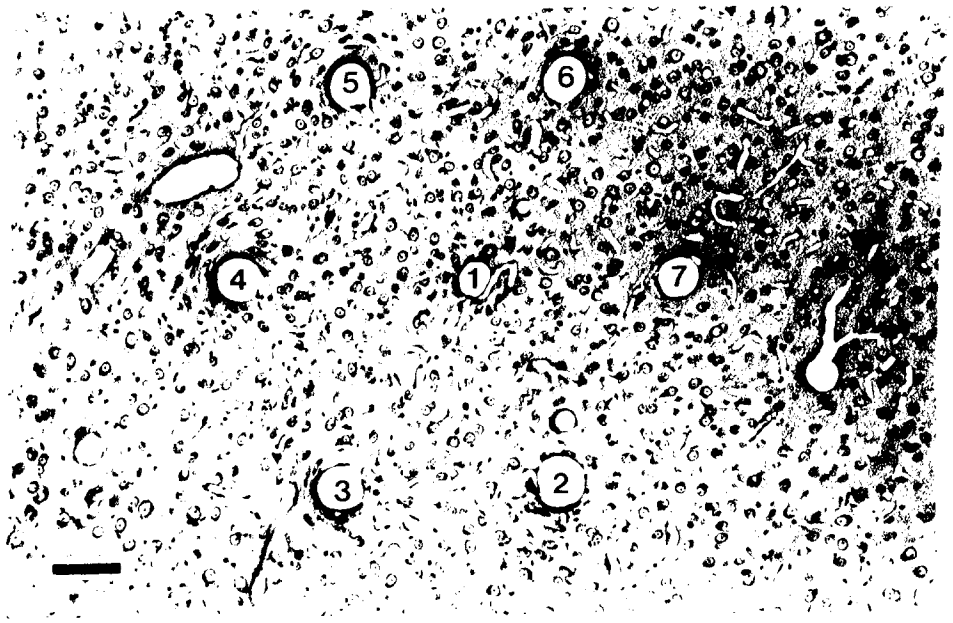

Fig. 1. IC166. All 7 electrode tracks are present in this horizontal section of the left postcruciate gyrus (depth 230 μm). The sheaths are 2-5 μm in thickness. Tracks 5 and 6 are the most anterior. Nissl stain. Bar = 50 μm.

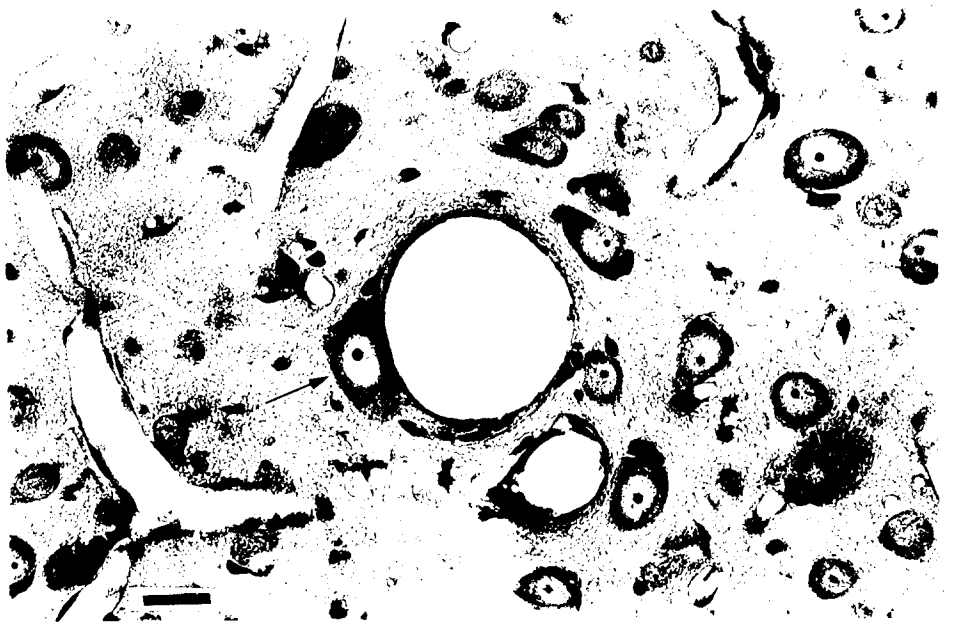

Fig. 2. IC166. Track 5 at a depth of 430 μm. Note the remarkably thin sheath and normal-appearing neurons and blood vessels nearby. One neuron (arrow) lies immediately adjacent to the track. Nissl stain. Bar = 25 μm.

A
B
C
D